17. (Amended) The method of claim 16, wherein the polynucleotide encoding the first G protein coupled receptor fusion protein and the polynucleotide encoding the second G protein coupled receptor fuson protein are homologous.

a8

18. (Amended) The method of claim 16, wherein the polynucleotide encoding the first G protein coupled receptor fusion protein and the polynucleotide encoding the second G protein coupled receptor fuson protein are heterologous.

a9

20. (Amended) The method of claim 16, wherein the polynucleotide encoding the first G protein coupled receptor encodes a truncated G protein coupled receptor.

Please add the following new claims:

27. (New) The method of claim 1, wherein the polynucleotide encoding the second G protein coupled receptor encodes a truncated G protein coupled receptor.

28. (New) The method of claim 6, wherein the polynucleotide encoding the second G protein coupled receptor encodes a truncated G protein coupled receptor.

a10

29. (New) The method of claim 11, wherein the polynucleotide encoding the second G protein coupled receptor encodes a truncated G protein coupled receptor.

30. (New) The method of claim 16, wherein the polynucleotide encoding the second G protein coupled receptor encodes a truncated G protein coupled receptor.

REMARKS

Claims 1-20 are pending in the present application. Claims 1-3, 5-8, 10-13, 15-18 and 20 are amended herein for clarity and to more particularly define the invention. Support for amended claims 1, 6, 11 and 16 can be found in claims 1, 6, 11 and 16 as filed, in the specification, on page 8, lines 15-18 and elsewhere throughout the specification. Support for

amended claims 2-3, 7-8, 12-13 and 17-18 can be found in claims 2-3, 7-8, 12-13 and 17-18 as originally filed and in the specification on page 7, lines 4-8. Support for amended claims 5, 10, 15 and 20 can be found in claims 1, 5, 6, 10, 11, 15, 16 and 20 as filed as well as throughout the specification. Support for new claims 27-30 can be found in claims 1, 5, 6,10, 11,15, 16 and 20 as filed and throughout the specification. It is believed that no new matter has been added by these amendments. In light of these amendments and the following remarks, applicants respectfully request reconsideration of this application, entry of the new claims and allowance of the claims to issue.

I. Objection to the Specification

A. The Office Action states that the specification is objected to since the title of the invention is allegedly not descriptive. The Examiner has suggested the following title: "Methods of detecting and modulating oligomerization of G protein-coupled receptors."

As suggested by the Examiner, the title has been amended to recite: "Methods of detecting and modulating oligomerization of G protein-coupled receptors." Therefore, applicants believe this objection has been overcome and respectfully request its withdrawal.

B. The Office Action also states that the specification is further objected to since the Brief Description of the Figures does not provide a brief description of Figure 4. Similarly, according to the Office Action, the Brief Description of the Figures also discusses Figure 3c, which is not present in the Figures themselves. It appears from the Brief Description of the Figures that Figure 4 should actually be labeled as Figure 3c. This would then be consistent with the Brief Description of the Figures which discusses parts a-c of Figure 3 and does not discuss Figure 4. Further evidence that Figure 4 should be renumbered as Figure 3c is due to the fact that there is a "c" on the page labeled "Figure 4," implying that this is actually panel "c" of Figure 3.

As suggested by the Examiner, Figure 4 should be labeled Figure 3c. Therefore, a replacement for Figure 4, now correctly labeled as Figure 3c is provided herein. Thus, applicants believe this objection has been overcome and respectfully request its withdrawal.

C. The Office Action further states that the specification is objected to since references 8 and 10 are missing from the "References" section on page 29 of the disclosure.

The specification is amended herein to renumber the references in the References list and to properly refer to these references throughout the specification. Thus, applicants believe this rejection has been overcome and respectfully request its withdrawal.

II. Rejection Under 35 U.S.C. § 112, first paragraph

A. The Office Action states that claims 11-20 are rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. According to the Office Action, in order to understand this rejection, claim 6 needs to be understood. Claim 6 recites that it can be determined whether an agonist activates G protein-coupled receptors (GPCRs) by either enhancing or disrupting oligomerization since, if the efficiency of FRET detected in cells contacted with an agonist is greater than that detected in cells prior to contact with said agonist, then receptor activation has occurred by enhancing oligomerization. Conversely, if the efficiency of FRET detected in cells contacted with an agonist is less than that detected in cells prior to contact with said agonist, then receptor activation has occurred by disrupting oligomerization. According to claim 6, the artisan is certain that the compound used in the method of this claim is, in fact, an agonist, and simply want to farther elucidate its mechanism of action with regard to oligomerization of GPCRs. However, this claim reveals that an agonist can activate G protein-coupled receptors by either enhancing or disrupting receptor oligomerization. Given this, according to the Office Action, the artisan, in screening for an unknown agonist or antagonist by performing the method of either claim 11 or 16 would notice one of three possible effects on FRET efficiency upon contacting the cells of the claim with the test since, again, according to claim 6 and page 2, lines 10-23 of the specification, an agonist could also disrupt (i.e. decreased or "less" FRET) oligomerization of GPCRs. Further stated in the Office Action is that claims 12-15 and 17-20 are rejected since they depend from rejected base claims.

Applicants agree with the Examiner that claim 6 recites a method of determining whether a known agonist activates G protein-coupled receptors (GPCRs) by either enhancing or disrupting oligomerization. In this regard, applicants respectfully point out that activation of G protein-coupled receptors is distinct from oligomerization of G protein-coupled receptors. As stated on page 7, lines 1-2 of the specification, "oligomerization" is defined as the association between two or more G protein coupled receptors. Oligomerization is the interaction between G protein-coupled receptors and is not equivalent to receptor activation, a process resulting in signal transduction via a receptor. As set forth in claim 6, a known agonist can exert its activating effects on a G protein-coupled receptor by enhancing oligomerization or by disrupting oligomerization. Therefore, it is clear that oligomerization is a mechanism by which an agonist can activate a receptor, but this mechanism is not equivalent to activation.

Furthermore, on page 12, lines 26-28 of the specification, applicants state that an agonist identified by the methods of the present invention can be assayed for signaling to determine whether or not the agonist identified in the assay exhibits biological properties similar to those of the receptor's natural ligand. Thus, it is clear that upon identifying an agonist of the interaction between G protein-coupled receptors (i.e. an agonist of oligomerization) one of skill in the art could also assay the agonist of the interaction between G protein-coupled receptors to determine if the agonist of oligomerization activates the receptors to produce signal transduction patterns or other biological properties similar to those effected by the receptor's natural ligand. Therefore, it is clear that an agonist of oligomerization does not necessarily activate G protein-coupled receptors.

Therefore, one of skill in the art could perform the method of claim 11 and identify an agonist of the interaction between G protein-coupled receptors by detecting FRET, such that if the efficiency of FRET detected is greater in cells contacted with the compound than the efficiency of FRET detected in cells prior to the addition of the test compound, the test compound is an agonist of the interaction between G protein coupled receptors. Upon obtaining this result, the skilled artisan would know that this compound is an agonist of the interaction between G protein-coupled receptors. In other words, the skilled artisan has identified a compound that enhances oligomerization, but this result does not indicate that the compound is

also an agonist of receptor activation. Since oligomerization and activation are distinct processes, further biological assays would be necessary in order to determine if and how this compound affects signal transduction or other processes associated with activation.

Similarly, one of skill in the art could perform the method of claim 16 and identify an antagonist of the interaction between G protein-coupled receptors by detecting FRET, such that if the efficiency of FRET detected is less than the efficiency of FRET detected in cells prior to the addition of the test compound, the test compound is an antagonist of the interaction between G protein coupled receptors. Upon obtaining this result, the skilled artisan would know that this compound is an antagonist of the interaction between G protein-coupled receptors. In other words, the skilled artisan has identified a compound that disrupts oligomerization, but this result does not indicate that the compound is also an antagonist of receptor activation. Since oligomerization and activation are distinct processes, further biological assays would be necessary in order to determine if and how this compound affects signal transduction or other processes associated with activation.

Therefore, claims 6, 11 and 16 accomplish different goals. More specifically, claim 6 is directed to determining whether or not a known agonist disrupts or enhances oligomerization; claim 11 is directed to identifying an agonist of the interaction between G protein-coupled receptors (i.e. a compound that enhances oligomerization), independent of whether or not this compound is also an agonist or an antagonist of G protein-coupled receptor activation; and claim 16 is directed to identifying an antagonist of the interaction between G protein-coupled receptors (i.e. a compound that disrupts oligomerization), independent of whether or not this compound is also an agonist or an antagonist of activation.

Therefore, claims 6, 11 and 16 are clearly enabled such that one of skill in the art can 1) determine a known agonist's mechanism of activation via oligomerization (claim 6); 2) identify an agonist of the interaction between G protein-coupled receptors (claim 11); and 3) identify an antagonist of the interaction between G protein-coupled receptors (claim 16). Thus, applicants believe that this rejection as it applies to claims 11-20 has been overcome and respectfully request its withdrawal.

B. The Office Action states that claims 16-20 are rejected under 35 U.S.C. 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Claim 16 recites that a test compound can be identified as an antagonist of the interaction between GPCRs if the FRET efficiency in cells contacted with the test compound is less than the FRET efficiency in said cells prior to contact with said test compound. According to the Office Action, the only logical way to interpret the method of claim 16 is that the antagonist would be reversing GPCR oligomerization, since this compound would be, according to the claim, decreasing FRET efficiency. Further stated in the Office Action is that this implies that the receptors were initially oligomerized and that FRET was detected in the absence of the test compound (antagonist). However, the Office Action contends that it is well-known to one of ordinary skill in the art that antagonists, by definition, do not produce any effect on a receptor. The Office Action states that the only "function" of an antagonist is to bind to a receptor to prevent, or reverse, the effects of an agonist, which does produce an effect on a receptor (Gilman AG. et al, The Pharmacological Basis of Therapeutics, 1993 - page 33, right column, second paragraph and page 45, right column, first paragraph). Therefore, according to the Office Action, based on this definition, a test compound which has the effect of decreasing FRET efficiency in a system is not an antagonist, but, in fact, an agonist and that the only way that the test compound identified in claim 16 could truly be considered an antagonist is if the claimed cells containing these GPCRs already contained an endogenous agonist for these GPCRs which allowed the initial oligomerization of these GPCRs. In this case, the artisan would observe an increase in FRET efficiency in the absence of a test compound due to the endogenous GPCR agonist in the cells. Therefore, according to the Office Action, any test compound which then decreased this agonist-induced FRET efficiency could then be considered an antagonist, since it would be competing with the agonist in the system to nullify the effect of said agonist and not producing any effect on its own. However, the Office Action further states that the claims as written do not enable the artisan to determine that the test compound is an antagonist. Claims 17-20 are rejected since they depend from rejected base claims.

Applicants respectfully point out to the Examiner that the apparent efficiencies of FRET observed with control cells treated with agonist or antagonist were indistinguishable (see page 25, lines 27-28 of the specification). Furthermore, applicants also showed that α-factor receptors are oligomeric during basal and agonist-induced internalization, in accord with results of FRET experiments showing that these receptors interact in the absence and presence of a receptor activation agonist (see page 27, lines 6-8 of the specification). Therefore, the present invention showed that G protein-coupled receptors oligomerize, *in vivo*, in intact cells, in the absence and presence of a receptor activation agonist. Therefore, a receptor activation agonist is not necessary for the initial oligomerization of G protein-coupled receptors and the method of claim 16 can be successfully performed in the absence of a receptor agonist to identify antagonists of the interaction between G protein-coupled receptors. Thus, applicants believe that claim 16 is adequately enabled and respectfully request withdrawal of this rejection.

III. Rejections Under 35 § 112, second paragraph

A. The Office action states that claims 1-20 are confusing since independent claims 1, 6, 11 and 16 recite "transfecting a cell with G protein-coupled receptor fusion proteins." However, cells are transfected with nucleic acid molecules encoding these fusion proteins and not with the fusion proteins themselves (see page 8, line 14- page 9, line 15 and the Example on page 22, line 18 - page 24, line 16 of the specification). Furthermore, according to the Office Action, the term "obtaining" does not add to the meaning of the claim since it is understood that in order to "transfect" a cell with (nucleic acid encoding) G protein coupled receptor fusion proteins, the artisan would need to have already obtained these fusion proteins, or DNA encoding these fusion proteins. Therefore, parts (a) - (c) of claims 1,6,11 and 16 could be reworded, for example, as follows:

a) transfecting a cell with:

   i) a polynucleotide encoding a first G protein-coupled receptor containing a fluorescence donor, and;

   ii) a polynucleotide encoding a second G protein-coupled receptor containing a fluorescence acceptor;

b) exciting the fluorescence donor...

As suggested by the Examiner, claim 1 is amended herein to recite a method of detecting oligomerization of G protein coupled receptors comprising: a) transfecting a cell with a polynucleotide encoding a first G protein-coupled receptor fusion protein containing a fluorescence acceptor and a polynucleotide encoding a second G protein-coupled receptor fusion protein containing a fluorescence donor; b) exciting the fluorescence donor at a particular wavelength; and c) detecting fluorescence emission of the acceptor (FRET), such that if this emission is greater than the emission detected in control cells expressing only the acceptor, oligomerization of the G protein coupled receptors has been detected.

Similarly, claim 6 is amended herein to recite a method of determining whether a receptor agonist activates G protein coupled receptors by enhancing oligomerization or activates G protein coupled receptors by disrupting oligomerization comprising: a) transfecting a cell with a polynucleotide encoding a first G protein-coupled receptor fusion protein containing a fluorescence acceptor and a polynucleotide encoding a second G protein-coupled receptor fusion protein containing a fluorescence donor; b) contacting the cell with an agonist; c) exciting the fluorescence donor at a particular wavelength; and d) detecting fluorescence resonance energy transfer (FRET) such that if the efficiency of FRET detected is greater in the cells contacted with the agonist than the efficiency of FRET detected in cells prior to the addition of the agonist, receptor activation has occurred by enhancing oligomerization and if the efficiency of FRET detected is less in the cells contacted with the agonist than the efficiency of FRET detected in cells prior to the addition of the agonist, receptor activation has occurred by disrupting oligomerization.

Also, claim 11 is amended herein to recite a method of screening for an agonist of the interaction between G protein coupled receptors comprising: a) transfecting a cell with a polynucleotide encoding a first G protein-coupled receptor fusion protein containing a fluorescence acceptor and a polynucleotide encoding a second G protein-coupled receptor fusion protein containing a fluorescence donor; b) contacting the cell with a test compound; c) exciting the fluorescence donor at a particular wavelength; and d) detecting fluorescence resonance energy transfer (FRET) such that if the efficiency of FRET detected is greater in the cells contacted with the compound than the efficiency of FRET detected in cells prior to the addition

of the test compound, the test compound is an agonist of the interaction between G protein coupled receptors.

Claim 16 is amended herein to recite a method of screening for an antagonist of the interaction between G protein coupled receptors comprising: a) transfecting a cell with a polynucleotide encoding a first G protein-coupled receptor fusion protein containing a fluorescence acceptor and a polynucleotide encoding a second G protein-coupled receptor fusion protein containing a fluorescence donor; b) contacting the cell with a test compound; c) exciting the fluorescence donor at a particular wavelength; and d) detecting fluorescence resonance energy transfer (FRET) such that if the efficiency of FRET detected is less than the efficiency of FRET detected in cells prior to the addition of the test compound, the test compound is an antagonist of the interaction between G protein coupled receptors.

Thus, applicants believe that this rejection has been overcome and respectfully request its withdrawal as it applies to claims 1, 6, 11 and 16 and their dependent claims.

B. The Office Action states that claims 2, 3, 5, 7, 8, 10, 12, 13, 15, 17, 18 and 20 are confusing since it is not clear as to what "receptor regions" the claims are referring. Furthermore, in claims 2, 3, 7, 8, 12, 13, 17 and 18, it is not understood what is meant by the recitation of the receptor regions of the fusion protein being the "same" or "different." Again, it is not clear as to what "receptor regions" the claims are referring. If the claims are intended to differentiate between homodimers (i.e. "same") and heterodimers (i.e. "different"), as discussed on page 7, lines 4-8 of the specification, then the term "region" should be removed and the claims should be amended to recite that the G protein-coupled receptors are either "homologous," (claims 2, 7, 12 and 17) or "heterologous" (claims 3, 8, 13 and 18). It is suggested that the term "regions" be removed from the claims.

As suggested by the Examiner, claim 2 is amended herein to recite the method of claim 1 wherein the polynucleotide encoding the first G protein coupled receptor fusion protein and the polynucleotide encoding the second G protein coupled receptor fusion protein are homologous. Also, Claim 3 is amended herein to recite the method of claim 1 wherein the polynucleotide

encoding the first G protein coupled receptor fusion protein and the polynucleotide encoding the second G protein coupled receptor fusion protein are heterologous. Claim 5 is also amended herein to recite the method of claim 1, wherein the polynucleotide encoding the first G protein coupled receptor fusion protein encodes a truncated G protein coupled receptor. Claims 7, 8, 10, 12, 13, 15, 17, 18 and 20 are similarly amended to incorporate the Examiner's suggestions. Thus, applicants believe this rejection has been overcome and respectfully request its withdrawal.

C. Also stated in the Office Action is that claims 5,10,15 and 20 are confusing since it is not clear which fusion protein is to be truncated, since there are two fusion proteins of the invention.

Claims 5, 10, 15 and 20 are amended herein to clarify which fusion protein is to be truncated. Claims 27-30 are added herein to further clarify the invention in this regard. Thus, applicants believe this rejection has been overcome and respectfully request its withdrawal.

D. The Office Action states that claims 11-15 are confusing since part (f) of claim 11 recites that it can be determined that a test compound is an agonist of GPCR interaction if the FRET efficiency in cells contacted with the test compound is greater than the FRET efficiency in said cells prior to contact with said test compound. This would, according to claim 6 and page 2, lines 10-23 of the specification, reveal that the agonist was acting by enhancing oligomerization of the G protein-coupled receptors. However, while it is understood how a test compound could be considered an agonist by enhancing oligomerization of GPCRs, according to claim 6 and page 2, lines 10-23 of the specification, an agonist could also disrupt oligomerization of GPCRs. This would be determined if the efficiency of FRET detected in cells contacted with an agonist is less than that detected in cells prior to contact with said agonist. However, it would not be clear to the artisan performing the method of claim 11 that, if the efficiency of FRET detected in cells contacted with a test compound is less than that detected in cells prior to contact with said test compound, that said test compound was not still, in fact, an agonist, since, again, according to claim 6 and page 2, lines 10-23 of the specification, an agonist could also disrupt (i.e. decreased

or "less" FRET) oligomerization of GPCRs. Claims 12-15 are rejected since they depend from rejected claim 11.

As stated above, claim 6 recites a method of determining whether a known agonist activates G protein-coupled receptors (GPCRs) by either enhancing or disrupting oligomerization. In this regard, applicants respectfully point out that activation of G protein-coupled receptors is distinct from oligomerization of G protein-coupled receptors. As stated on page 7, lines 1-2 of the specification, "oligomerization" is defined as the association between two or more G protein coupled receptors. Oligomerization is the interaction between G protein-coupled receptors and is not equivalent to receptor activation, a process resulting in signal transduction via a receptor. As set forth in claim 6, a known agonist can exert its activating effects on a G protein-coupled receptor by enhancing oligomerization or by disrupting oligomerization. Therefore, it is clear that oligomerization is a mechanism by which an agonist can activate a receptor, but this mechanism is not equivalent to activation.

Furthermore, on page 12, lines 26-28 of the specification, applicants state that an agonist identified by the methods of the present invention can be assayed for signaling to determine whether or not the agonist identified in the assay exhibits biological properties similar to those of the receptor's natural ligand. Thus, it is clear that upon identifying an agonist of the interaction between G protein-coupled receptors (i.e. an agonist of oligomerization) one of skill in the art could also assay the agonist of the interaction between G protein-coupled receptors to determine if the agonist of oligomerization activates the receptors to produce signal transduction patterns or other biological properties similar to those effected by the receptor's natural ligand. Therefore, it is clear that an agonist of oligomerization does not necessarily activate G protein-coupled receptors.

Therefore, one of skill in the art could perform the method of claim 11 and identify an agonist of the interaction between G protein-coupled receptors by detecting FRET, such that if the efficiency of FRET detected is greater in cells contacted with the compound than the efficiency of FRET detected in cells prior to the addition of the test compound, the test compound is an agonist of the interaction between G protein coupled receptors. Upon obtaining

this result, the skilled artisan would know that this compound is an agonist of the interaction between G protein-coupled receptors. In other words, the skilled artisan has identified a compound that enhances oligomerization, but this result does not indicate that the compound is also an agonist of receptor activation. Since oligomerization and activation are distinct processes, further biological assays would be necessary in order to determine if and how this compound affects signal transduction or other processes associated with activation.

Therefore, claims 6 and 11 accomplish different goals. More specifically, claim 6 is directed to determining whether or not a known agonist disrupts or enhances oligomerization; and claim 11 is directed to identifying an agonist of the interaction between G protein-coupled receptors (i.e. a compound that enhances oligomerization), independent of whether or not this compound is also an agonist or an antagonist of activation. Thus, claims 6 and 11 are clearly described such that one of skill in the art can 1) determine a known agonist's mechanism of activation via oligomerization (claim 6); and 2) identify an agonist of the interaction between G protein-coupled receptors (claim 11). Thus, applicants believe that this rejection as it applies to claims 11-15 has been overcome and respectfully request its withdrawal.

E. Further stated in the Office Action is that claims 16-20 are confusing since part (f) of claim 16 recites that it can be determined that a test compound is an antagonist of GPCR interaction if the FRET efficiency in cells contacted with the test compound is less than the FRET efficiency in said cells prior to contact with said test compound. However, according to claim 6 and page 2, lines 10-23 of the specification, agonists of GPCR interaction can also act by disrupting oligomerization of the G protein-coupled receptors, since the efficiency of FRET detected in cells contacted with an agonist would also be less than that detected in cells prior to contact with said agonist. Therefore, according to the Office Action, it would not be clear to the artisan performing the method of claim16 that, if the efficiency of FRET detected in cells contacted with a test compound is less than that detected in cells prior to contact with said test compound, that said test compound which, according to the method of claim 16 would identify an antagonist, was not still, in fact, an agonist. Claims 17-20 are rejected since they depend from rejected claim 16.

As stated above, claim 6 recites a method of determining whether a known agonist activates G protein-coupled receptors (GPCRs) by either enhancing or disrupting oligomerization. In this regard, applicants respectfully point out that activation of G protein-coupled receptors is distinct from oligomerization of G protein-coupled receptors. As stated on page 7, lines 1-2 of the specification, "oligomerization" is defined as the association between two or more G protein coupled receptors. Oligomerization is the interaction between G protein-coupled receptors and is not equivalent to receptor activation, a process resulting in signal transduction via a receptor. As set forth in claim 6, a known agonist can exert its activating effects on a G protein-coupled receptor by enhancing oligomerization or by disrupting oligomerization. Therefore, it is clear that oligomerization is a mechanism by which an agonist can activate a receptor, but this mechanism is not equivalent to activation.

Furthermore, on page 12, lines 26-28 of the specification, applicants state that an agonist identified by the methods of the present invention can be assayed for signaling to determine whether or not the agonist identified in the assay exhibits biological properties similar to those of the receptor's natural ligand. Thus, it is clear that upon identifying an agonist of the interaction between G protein-coupled receptors (i.e. an agonist of oligomerization) one of skill in the art could also assay the agonist of the interaction between G protein-coupled receptors to determine if the agonist of oligomerization activates the receptors to produce signal transduction patterns or other biological properties similar to those effected by the receptor's natural ligand. Therefore, it is clear that an agonist of oligomerization does not necessarily activate G protein-coupled receptors.

Therefore, one of skill in the art could perform the method of claim 16 and identify an antagonist of the interaction between G protein-coupled receptors by detecting FRET, such that if the efficiency of FRET detected is less than the efficiency of FRET detected in cells prior to the addition of the test compound, the test compound is an antagonist of the interaction between G protein coupled receptors. Upon obtaining this result, the skilled artisan would know that this compound is an antagonist of the interaction between G protein-coupled receptors. In other words, the skilled artisan has identified a compound that disrupts oligomerization, but this result does not indicate that the compound is also an antagonist of receptor activation. Since

oligomerization and activation are distinct processes, further biological assays would be necessary in order to determine if and how this compound affects signal transduction or other processes associated with activation.

Therefore, claims 6 and 16 accomplish different goals. More specifically, claim 6 is directed to determining whether or not a known receptor agonist disrupts or enhances oligomerization; and claim 16 is directed to identifying an antagonist of the interaction between G protein-coupled receptors (i.e. a compound that disrupts oligomerization), independent of whether or not this compound is also an agonist or an antagonist of activation.

Therefore, claims 6 and 16 are clearly described such that one of skill in the art can 1) determine a known agonist's mechanism of activation via oligomerization (claim 6); and 2) identify an antagonist of the interaction between G protein-coupled receptors (claim 16). Thus, applicants believe that this rejection as it applies to claims 16-20 has been overcome and respectfully request its withdrawal.

F. The Office Action states that Claims 16-20 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. More specifically, the Office Action states that clam 16 allegedly lacks the essential step of determining whether or not endogenous agonists to GPCRs exist in the cell.

As stated above, applicants showed that the apparent efficiencies of FRET observed with control cells treated with agonist or antagonist were indistinguishable (see page 25, lines 27-28 of the specification). Furthermore, applicants also showed that α-factor receptors are oligomeric during basal and agonist-induced internalization, in accord with results of FRET experiments showing that these receptors interact in the absence and presence of agonist (see page 27, lines 6-8 of the specification). Therefore, the present invention showed that G protein-coupled receptors oligomerize, *in vivo*, in intact cells, in the absence and presence of a receptor activation agonist. Therefore, a receptor activation agonist is <u>not</u> necessary for the initial oligomerization of G protein-coupled receptors and the method of claim 16 can be successfully performed in the

absence of a receptor activation agonist to identify antagonists of the interaction between G protein-coupled receptors. Thus, determining whether or not endogenous agonists to G protein coupled receptors exist in the cell is not a necessary step in the method of claim 16. Therefore, applicants believe that claim 16 is complete and respectfully request withdrawal of this rejection as it applies to claims 16-20.

IV. Rejections Under 35 U.S.C. § 103(a)

A. The Office Action states that claims 1-10 are rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over White et al. (reference AG on the IDS of Paper No.3) in view of Miyawaki et al. (Nature 388:882-887, 1997) and further in view of Hebert et al. (reference Al on the IDS of Paper No. 3). According to the Office Action, White et al. teach that homodimerization has been reported for several seven transmembranereceptors (i.e. GPCRs), including the δ-opioid, β2-adrenergic and metabotropic glutamate receptors (page 682, left column, first paragraph). White et al. also teach that G protein-coupled GABABR2 (R2) receptors form heterodimers with G protein-coupled $GABA_BRI$ (Rl) receptors (Abstract and Figure 2b). Therefore, White et al. teach that both homodimerization and heterodimerization of GPCRs does occur and that this oligomerization (protein-protein interaction) is required for receptor activity. This can be seen in Figure 2a on page 680 of White et al. where neither Rl, nor R2 are able to increase β- galactosidase activity either separately with only empty vector controls, or when attempting to be expressed as homodimers, but only when Rl and R2 are co-expressed. White et al. also teach that the known receptor agonist, GABA, is able to stimulate $[^{35}S]GTP\gamma S$ binding in cells expressing both R2 and Rl receptors, but not in cells expressing only one of these receptors (Figure 4b). According to the Office Action, this experiment provides further evidence that GABA receptors oligomerize (dimerize). However, White et al. do not teach the use of FRET to detect this oligomerization, regardless of whether or not the GPCR regions are the same, or different, or whether the receptor region of the GPCR fusion protein is truncated, nor do they teach the use of cyan fluorescence protein or yellow fluorescence protein. Furthermore, White et al. do not teach a method of determining whether a receptor agonist activates GPCRs by enhancing or disrupting oligomerization of these GPCRs. However, Miyawaki et al. do teach the use of fusing the fluorescence indicators cyan fluorescence protein (CFP) and yellow fluorescence protein (YFP) to the non-GPCR calmodulin and MIS proteins,

respectively (page 887 under "Gene construction"), in order to monitor protein heterodimerization, as well as to monitor the effect of Ca2+-induced conformational changes between the protein-protein interactions of cahnodulin and MIS (Abstract). CFP was used as the donor and YFP was used as the acceptor (Figure la-c; page 885, right column, first fall paragraph; page 886, right column, first fall paragraph). In addition, Miyawaki et al. teach that, as expected, Ca2+, acts as an agonist by inducing a conformational change in the protein-protein interaction of calmodulin and MIS (Figure la) as detected by an increased efficiency of FRET in response to increasing CaZ' concentrations (Figure 2a).

To summarize, White et al. teach that GPCRs can oligomerize by forming either homodimers (i.e. same regions) or heterodimers (i.e. different regions). This protein-protein interaction taught by White et al. is also taught by Miyawaki et al. since Miyawaki et al. teach that heterodimerization of calmodulin and MIS proteins occurs. Miyawaki et al. teach that this dimerization of calmodulin and MIS can be detected by FRET using calmodulin and MIS fusion proteins fused to fluorescent proteins including CFP and YFP. Therefore, given the teachings of oligomerization of the GPCRs of White et al. and the FRET assay to observe the oligomerization of the proteins of Miyawaki et al, it would have been obvious to one of ordinary skill in the art at the time of the present invention to have produced GPCR fusion proteins in which a first GPCR was fused to a fluorescence donor, such as CFP, and a second GPCR, which was either the same as (i.e. homologous) or different than (i.e. heterologous) said first GPCR, was fused to a fluorescence acceptor, such as YFP, for the purpose of detecting oligomerization of GPCRs using this very specific and sensitive FRET assay. The only difference between the proteins of White et al. and those of Miyawaki et al. is that the proteins of Miyawaki et al. are not GPCRs. However, given that the oligomerization of calmodulin and MIS proteins can be detected using FRET, the artisan would have been motivated to produce GPCR-fluorescent fusion proteins to detect oligomerization using FRET since it was well-known at the time of the invention that GPCRs oligomerize (White et al.). In fact, Hebert et al.,who teach that GPCRs oligomerize, even state that one of the next steps that is required in demonstrating that GPCRs for biologically regulated dimmers in whole cells is to use a real-time measurement assay such as fluorescence resonance energy transfer (FRET; page 8, last paragraph). Furthermore, given that Miyawaki et al. detected the oligomerization of two proteins using FRET, there would also have

been a reasonable expectation of success for one of ordinary skill in the art at the time of the present invention to have detected the oligomerization of the two proteins of White et al. Furthermore, there would have been a reasonable expectation of success in producing the GPCR-fusion proteins of White et al. using the teachings of Miyawaki et al. since there is no difference in the recombinant techniques used to produce fusion proteins between either GPCRs and fluorescent proteins and non-GPCRs and fluorescent proteins. In addition, it would have also have been obvious to one of ordinary skill in the art at the time of the invention to have determined whether a receptor agonist activated GPCR by enhancing or disrupting oligomerization since both Miyawaki et al. and White et al. teach that agonists affect the function of oligomers. Miyawaki et al. show that Ca2+ acts as an agonist for the calmodulin-MIS interaction by increasing FRET efficiency, as diagrammed in Figure la. Similarly, White et al. demonstrate that the known agonist, GABA, stimulates [^{35}S]GTPγS binding in cells containing both R2 and Rl receptors. Therefore, the artisan would have been able to detect either an increased or decreased FRET efficiency compared to cells prior to the addition of an agonist since these would have been. the proper basis control studies to have performed by one of ordinary skill in the art. The artisan would have been motivated to have used a FRET assay in order to monitor the oligomerization of the GPCRs of White et al. since the use of FRET has several advantages over covalent labeling with fluorescent probes. First, it is a non-destructive spectroscopic method which combines the brightness of fluorescence indicators with the targeting ability of a biosynthetic indicator in which the indicator is generated in situ by gene transfer into the cells or organism, obviating the need for large-scale purification, labeling and microinjection of recombinant proteins that must be soluble. Second, the sites of the fusions are exactly defined, providing a homogenous product. Third, the chromophore is fixed into the protein, as opposed to the use of a flexible linker which partly decouples the fluorophore orientation from the protein to which it is attached (Miyawaki et al. page 883, top of left column). Claims 5 and 10 are rejected since they depend from rejected claims 1 and 6, respectively.

Applicants respectfully point out to the Examiner that the present invention provides the first real time, *in vivo* evidence of G protein-coupled receptor oligomerization in intact cells. Until the present invention, accurate, meaningful *in vivo* measurements of the oligomerization

between G protein-coupled receptors in intact cells was not possible. Therefore, *in vivo* screening methods for identifying compounds that affected G protein-coupled receptor oligomerization were not available. Thus, this invention also provides the first real time, *in vivo* screening methods for identifying compounds that affect oligomerization of G protein-coupled receptors. The ability to directly measure the oligomerization of G protein-coupled receptors and the effects of compounds on the oligomerization of G protein-coupled receptors in intact cells, allows a better understanding of how receptors interact *in vivo* as well as a better understanding of how compounds that affect these receptors exert their effects *in vivo*.

According to the Office Action, White et al., teach that G protein-coupled receptors can oligomerize by forming either homodimers or heterodimers. Applicants respectfully point out that the receptor interactions mentioned by White et al, including the receptor interactions of the GABA receptors studied by White et al, are based on *in vitro* results. For example, White et al. describes the use of a yeast two-hybrid system to show that $GABA_BR1$ and $GABA_BR2$ interact (page 680, col. 1, second full paragraph). This interaction is not evidence of an *in vivo* interaction between these receptors, on the cell membrane of intact cells. Therefore, the fact that neither Rl, nor R2 are able to increase β- galactosidase activity either separately with only empty vector controls, or when attempting to be expressed as homodimers, but only when Rl and R2 are co-expressed in a yeast two hybrid assay is not indicative of how these receptors interact, *in vivo*, in intact cells. With regard to White et al.'s teaching that the known receptor agonist, GABA, is able to stimulate $[^{35}S]GTP\gamma S$ binding in cells expressing both R2 and Rl receptors, but not in cells expressing only one of these receptors, applicants point out that this result is indicative of an agonist potentially interacting with two receptors to exert its effects, but not necessarily indicative of *in vivo* oligomerization between the receptors on the cell membrane.

White et al. also describes the coimmunoprecipitation experiments in which cell surface expression of $GABA_BR1$ was observed only when $GABA_BR2$ was also expressed, and vice versa. The coimmunoprecipitation experiments also showed that both receptors could be precipitated together after coexpression. Once again, these *in vitro* results are only indicative of an interaction between $GABA_BR1$ and $GABA_BR2$, but this interaction is not evidence of any *in vivo* interaction between the receptors in intact cells, much less a meaningful *in vivo* interaction

that allows direct measurement of receptor interaction and screening for compounds that affect this interaction. In summary, White et al., provides only indirect evidence of receptor oligomerization based on *in vitro* results and not definitive evidence that *in vivo* receptor interactions (i.e. oligomerization) occur on the cell membrane, in intact cells, in such a way, that these interactions can be observed, in real time, and accurately measured.

Although Miyawaki et al. teaches the use of FRET for monitoring protein-protein interactions, the proteins utilized by Miyawaki et al. were calmodulin and M13, neither one of which is a G protein coupled receptor. Although Miyawaki et al. utilized FRET to show that these proteins interact and that the efficiency of FRET between them can be increased by an agonist, the teachings of Miyawaki et al., do not provide any guidance or suggestion that G protein-coupled receptors fused to fluorescent donors and acceptors can be efficiently expressed on the cell membrane such that their interaction can be measured. Furthermore, although the Office Action states that Hebert et al. suggests that one of the next steps that required in demonstrating G protein-coupled receptors for biologically regulated dimers in whole cells is to use a real-time measurement assay such as FRET, this reference merely provides a suggestion and it was not until applicants actually obtained meaningful expression resulting in accurate, measurable interactions that FRET could be effectively utilized to study the interaction between G protein-coupled receptors. As stated above, such real time, *in vivo* interactions, in intact cells, provide the basis for the screening methods claimed in the present application. Without this breakthrough, the effects of compounds on receptor oligomerization in intact cells, and more importantly, the effects of compounds on receptor oligomerization, *in vivo*, could not be studied.

Thus, applicants believe that the present invention provides a significant advance in the field of receptor biology. Although it might have been obvious to try FRET experiments with G protein-coupled receptors, the ability to measure receptor interactions was not possible until applicants definitively showed that G protein-coupled receptors interact *in vivo*, in intact cells. In fact, applicants' work has been cited for their original use of FRET to show homodimerization in living cells. (see Bouvier "Oligomerization of G-Protein Coupled Transmitter Receptors" *Nat Rev Neurosci* 2:274-8 (2001), specifically page 276, second col. first full paragraph) (attached hereto). Therefore, applicants believe that the present invention is not obvious over White et al.,

in view of Miyawaki et al. and further in view of Hebert et al. Thus, applicants believe this rejection has been overcome and respectfully request its withdrawal.

B. The Office Action states that claims 5 and 10 are rejected under 35 U.S.C. 103(a) as being unpatentable over White et al. (reference AG on the IDS of Paper No.3) in view of Miyawaki et al. (Nature 388:882-887, 1997), further in view of Gama et al. (J. Biol. Chem. 273:29712-29718, 1998) and further in view of Hebert et al.

The teachings of White et al., Hebert et al., and Miyawaka et al. are discussed above. The Office Action states that Gama et al., teach that calcium sensing receptors (CaRs), which are GPCRs, can be truncated. In addition, Gama et al. produced fusion proteins comprising these truncated CaRs by combining the truncated CaRs with green fluorescent proteins (GFP) in order to assess any alterations in Ca2+-dependent activation or desensitization (Abstract). The Office Action states that Gama et al. not only demonstrate that truncated CaRs fused with GFP are functional (Table I, on page 29716), but that GFP can be used to visualize the location and movement of these truncation-mutant fusion proteins (Figure 4). According to the Office Action, it would have been obvious to one of ordinary skill in the art at the time of the present invention to have produced the truncated GPCR of Gama et al. and to have coupled this protein to a fluorescent fusion protein for use in a FRET assay since, not only have fluorescent fusion proteins comprising GPCRs (i.e. CaR) been produced, but Gama et al. also teach that CaRs represent a novel member of a GPCR family which includes metabotropic glutamate receptors (mGluRs; page 29712, right column, first full paragraph).

As stated above, the receptor interactions mentioned by White et al, including the receptor interactions of the GABA receptors studied by White et al, are based on *in vitro* results. Therefore, White et al., provides only indirect evidence of receptor oligomerization based on *in vitro* results and not definitive evidence that *in vivo* receptor interactions (i.e. oligomerization) occur on the cell membrane, in intact cells, in such a way, that these interactions can be observed, in real time, and accurately measured. In fact, the art has recognized that effects observed *in vitro*, can, in fact, be artifacts (see Bouvier "Oligomerization of G-Protein Coupled

Transmitter Receptors" *Nat Rev Neurosci* 2:274-8 (2001), specifically page 276, first col. last paragraph) (attached hereto).

Also, as stated above, although Miyawaki et al. teaches the use of FRET for monitoring protein-protein interactions, the proteins utilized by Miyawaki et al. were calmodulin and M13, neither one of which is a G protein coupled receptor. Although Miyawaki et al. utilized FRET to show that these proteins interact and that the efficiency of FRET between them can be increased by an agonist, the teachings of Miyawaki et al., do not provide any guidance or certainty that G protein-coupled receptors fused to fluorescent donors and acceptors can be efficiently expressed on the cell membrane such that their interaction can be measured. Furthermore, although the Office Action states that Hebert et al. suggests that one of the next steps that required in demonstrating G protein-coupled receptors for biologically regulated dimers in whole cells is to use a real-time measurement assay such as FRET, this reference merely provides a suggestion and it was not until applicants actually obtained meaningful expression resulting in accurate, measurable interactions that FRET could be effectively utilized to study the interaction between G protein-coupled receptors. As stated above, such real time, *in vivo* interactions, in intact cells, provide the basis for the screening methods claimed in the present application. Without this breakthrough, the effects of compounds on receptor oligomerization in intact cells, and more importantly, the effects of compounds on receptor oligomerization, *in vivo*, could not be studied.

Furthermore, although the Office Action contends that it would have been obvious to one of ordinary skill in the art at the time of the present invention to have produced the truncated G protein-coupled receptors of Gama et al. and to have coupled this protein to a fluorescent fusion protein for use in a FRET assay, the GFP fusion proteins of Gama et al. do not provide any indication as to how fluorescent fusion proteins comprising G protein-coupled receptors can be utilized in a FRET assay. Furthermore, Gama et al. provides no indication that the interaction between G protein-coupled receptors can be measured. Therefore, there is no suggestion or motivation provided by Gama et al. to study the interactions between G protein-coupled receptors.

Thus, applicants believe that the present invention provides a significant advance in the field of receptor biology. Although it might have been obvious to try FRET experiments with G protein-coupled receptors, the ability to measure receptor interactions was not possible until applicants definitively showed that G protein-coupled receptors interact *in vivo*, in intact cells. Therefore, applicants believe that the present invention is not obvious over White et al., in view of Miyawaki et al., further in view of Gama et al. and further in view of Hebert et al. Thus, applicants believe this rejection has been overcome and respectfully request its withdrawal.

Pursuant to the above amendments and remarks, reconsideration and allowance of the pending claims in this application is believed warranted. The Examiner is invited and encouraged to directly contact the undersigned if such contact may enhance the efficient prosecution of this application to issue.

Payment in the amount of $496.00 ($460.00 extension of time fee and $36 for four (4) additional dependent claims is to be charged to a credit card and such payment is authorized by the signed, enclosed document entitled Credit Card Payment Form PTO-2038. This amount is believed to be correct; however, the Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

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CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231, on the date shown below.

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11-7-02

Date

Marked-Up Version of Amendments
U.S. Application Serial No. 09/679,852

IN THE SPECIFICATION

The paragraph bridging pages 4 and 5:

Figure 2 shows the oligomerization of α-factor receptors during endocytosis. Fluorescence microscopy was used to localize GFP-tagged tailless receptors co-expressed with untagged wild type receptors (Δtail-GFP + WT) or alone (Δtail-GFP), GFP-tagged glucose transporters expressed with untagged wild type receptors (Hxt1-GFP + WT), and wild type receptors tagged with GFP (WT-GFP). Images were acquired before (0 min) or at the indicated times after the addition of agonist (α-factor, 5μM). Endosomal vesicles (E), the lysosome-like vacuole (V) and the endoplasmic reticulum (R) are indicated, as documented previously[(21)].

The paragraph bridging pages 24 and 25:

FRET experiments [(8)]were performed using truncated α-factor receptors lacking their cytoplasmic C-terminal regulatory domains (Ste2Δtail-CFP, donor; Ste2Δtail-YFP, acceptor), which are normal with respect to expression level, agonist binding affinity and G protein activation [(9)] (8). Truncated receptors were used to reduce interfluorophore distance or mobility, improving the likelihood of detecting FRET, and to eliminate phosphorylation and ubiquitination of the receptor, which are required for desensitization and endocytosis. This ensured that FRET would not detect interactions between desensitized or internalized receptors.

The first full paragraph on page 27:

If oligomerization is required for normal signaling, then cells expressing wild type receptors should signal inefficiently if they also overexpress signaling-defective receptors that interact with wild type receptors. Signal inhibition could occur if mutant receptor subunits interfere with the ability of wild type receptor subunits to undergo agonist induced conformational changes or to activate G protein heterotrimers. Accordingly, dominant-interfering mutant receptors were selected that inhibit signaling when overexpressed in cells expressing normal levels of wild type receptors [(10)]. This approach identified a novel

substitution (M250I) in transmembrane segment VI, a domain that controls activation of the α-factor receptor [(11)] (9).

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IN THE CLAIMS

1. (Amended) A method of detecting oligomerization of G protein coupled receptors comprising:

[a) obtaining a first G protein coupled receptor fusion protein containing a fluorescence donor;

b) obtaining a second G protein coupled receptor fusion protein containing a fluorescence acceptor;

c) transfecting a cell with the G protein coupled receptor fusion proteins of a) and b);]

a) transfecting a cell with a polynucleotide encoding a first G protein-coupled receptor fusion protein containing a fluorescence acceptor and a polynucleotide encoding a second G protein-coupled receptor fusion protein containing a fluorescence donor;

[d)] b) exciting the fluorescence donor at a particular wavelength;

[e)] c) detecting fluorescence emission of the acceptor (FRET), such that if this emission is greater than the emission detected in control cells expressing only the acceptor, oligomerization of the G protein coupled receptors has been detected.

2. (Amended) The method of claim 1, wherein the [first] polynucleotide encoding the first G protein coupled receptor fusion protein and the [second] polynucleotide encoding the second G protein coupled receptor fuson protein [G protein coupled receptor regions of the fusion protein] are [the same] homologous.

3. (Amended) The method of claim 1, wherein the [first] polynucleotide encoding the first G protein coupled receptor fusion protein and the [second] polynucleotide encoding the second G protein coupled receptor fuson protein [G protein coupled receptor regions of the fusion protein] are [different] heterologous.

5. (Amended) The method of claim 1, wherein the polynucleotide encoding the first G protein coupled receptor [region of the fusion protein is] encodes a truncated G protein coupled receptor.

6. (Amended) A method of determining whether a receptor agonist activates G protein coupled receptors by enhancing oligomerization or activates G protein coupled receptors by disrupting oligomerization comprising:

[a) obtaining a first G protein coupled receptor fusion protein containing a fluorescence donor;

b) obtaining a second G protein coupled receptor fusion protein containing a fluorescence acceptor;

c) transfecting a cell with the G protein coupled receptor fusion proteins of a) and b);]

a) transfecting a cell with a polynucleotide encoding a first G protein-coupled receptor fusion protein containing a fluorescence acceptor and a polynucleotide encoding a second G protein-coupled receptor fusion protein containing a fluorescence donor;

[d)] b) contacting the cell with an agonist;

[e)] c) exciting the fluorescence donor at a particular wavelength;

[f)] d) detecting fluorescence resonance energy transfer (FRET), such that if the efficiency of FRET detected is greater in the cells contacted with the agonist than the efficiency of FRET detected in cells prior to the addition of the agonist, receptor activation has occurred by enhancing oligomerization and if the efficiency of FRET detected is less in the cells contacted with the agonist than the efficiency of FRET detected in cells prior to the addition of the agonist, receptor activation has occurred by disrupting oligomerization.

7. (Amended) The method of claim 6, wherein the [first] polynucleotide encoding the first G protein coupled receptor fusion protein and the [second] polynucleotide encoding the second G protein coupled receptor fuson protein [G protein coupled receptor regions of the fusion protein] are [the same] homologous.

8. (Amended) The method of claim 6, wherein the [first] polynucleotide encoding the first G protein coupled receptor fusion protein and the [second] polynucleotide encoding the second G protein coupled receptor fuson protein [G protein coupled receptor regions of the fusion protein] are [the same] heterologous.

10. (Amended) The method of claim 6, wherein the polynucleotide encoding the first G protein coupled receptor [region of the fusion protein is] encodes a truncated G protein coupled receptor.

11. (Amended) A method of screening for an agonist of the interaction between G protein coupled receptors comprising:

[a) obtaining a first G protein coupled receptor fusion protein containing a fluorescence donor;

b) obtaining a second G protein coupled receptor fusion protein containing a fluorescence acceptor;

d) transfecting a cell with the G protein coupled receptor fusion proteins of a) and b);]

a) transfecting a cell with a polynucleotide encoding a first G protein-coupled receptor fusion protein containing a fluorescence acceptor and a polynucleotide encoding a second G protein-coupled receptor fusion protein containing a fluorescence donor;

[d)] b) contacting the cell with a test compound;

[e)] c) exciting the fluorescence donor at a particular wavelength;

[f)] d) detecting fluorescence resonance energy transfer (FRET), such that if the efficiency of FRET detected is greater in cells contacted with the compound than the efficiency of FRET detected in cells prior to the addition of the test

compound, the test compound is an agonist of the interaction between G protein coupled receptors.

12. (Amended) The method of claim 11, wherein the [first] polynucleotide encoding the first G protein coupled receptor fusion protein and the [second] polynucleotide encoding the second G protein coupled receptor fuson protein [G protein coupled receptor regions of the fusion protein] are [the same] homologous.

13. (Amended) The method of claim 11, wherein the [first] polynucleotide encoding the first G protein coupled receptor fusion protein and the [second] polynucleotide encoding the second G protein coupled receptor fuson protein [G protein coupled receptor regions of the fusion protein] are [the same] heterologous.

15. (Amended) The method of claim 11, wherein the polynucleotide encoding the first G protein coupled receptor [region of the fusion protein is] encodes a truncated G protein coupled receptor.

16. (Amended) A method of screening for an antagonist of the interaction between G protein coupled receptors comprising:

[a) obtaining a first G protein coupled receptor fusion protein containing a fluorescence donor;

b) obtaining a second G protein coupled receptor fusion protein containing a fluorescence acceptor;

c) transfecting a cell with the G protein coupled receptor fusion proteins of a) and b);]

a) transfecting a cell with a polynucleotide encoding a first G protein-coupled receptor fusion protein containing a fluorescence acceptor and a polynucleotide encoding a second G protein-coupled receptor fusion protein containing a fluorescence donor;

[d)] b) contacting the cell with a test compound;

[e)] c) exciting the fluorescence donor at a particular wavelength;

[f)] d) detecting fluorescence resonance energy transfer (FRET), such that if the efficiency of FRET detected is less than the efficiency of FRET detected in cells prior to the addition of the test compound, the test compound is an antagonist of the interaction between G protein coupled receptors.

17. (Amended) The method of claim 16, wherein the [first] polynucleotide encoding the first G protein coupled receptor fusion protein and the [second] polynucleotide encoding the second G protein coupled receptor fuson protein [G protein coupled receptor regions of the fusion protein] are [the same] homologous.

18. (Amended) The method of claim 16, wherein the [first] polynucleotide encoding the first G protein coupled receptor fusion protein and the [second] polynucleotide encoding the second G protein coupled receptor fuson protein [G protein coupled receptor regions of the fusion protein] are [the same] heterologous.

20. (Amended) The method of claim 16, wherein the polynucleotide encoding the first G protein coupled receptor [region of the fusion protein is] encodes a truncated G protein coupled receptor.